IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Gorr et al.	Examiner: Anne Kubelik
Application Number: 10/089,450	Group Art Unit: 1638
Filed: March 29, 2002	
For: METHOD FOR PRODUCTION OF PROTEINACEOUS SUBSTANCES	

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

REPLY BRIEF

This Reply Brief is filed within two months of the Examiner's Answer mailed December 7, 2010.

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I

Status of the Claims

Claims 1-3, 17, 22 and 24 remain rejected pursuant to a Non-Final Action mailed September 27, 2009 and pursuant to the Examiner's Answer mailed December 7, 2010. Claims 1-3, 17, 22 and 24 remain subject of this appeal. The status of each claim is as follows:

Claims canceled:	4-16, 18-21 and 23
Claims withdrawn:	none
Claims allowed:	none
Claims rejected:	1-3, 17, 22 and 24
Claims pending:	1-3, 17, 22 and 24
Claims on appeal:	1-3, 17, 22 and 24

II

Grounds of Rejection to Be Reviewed on Appeal

The following grounds of rejection remain on appeal:

1. Did the Examiner err in concluding that claims 1-3, 17 and 24 were obvious under 35 U.S.C. §103(a) over 1996, Plant Tiss. Cult. Biotechnol. 2:142-147 published by Reutter et al. in view of U.S. Patent No. 6,096,546 issued to Raskin?

2. Did the Examiner err in concluding that claim 22 is obvious under 35 U.S.C. §103(a) over 1996, Plant Tiss. Cult. Biotechnol. 2:142-147 published by Reutter et al. in view of U.S. Patent No. 6,096,546 issued to Raskin and further in view of U.S. Patent No. 5,959,177 issued to Hein et al.

III

Argument

1. Claims 1-3, 17 and 24 remain rejected under 35 U.S.C. §103(a) as being unpatentable over Reutter et al. (1996, Plant Tiss. Cult. Biotechnol. 2:142-147) in view of Raskin (U.S. Patent No. 6,096,546).

Appellant respectfully disagrees with the Examiner's rejection and respectfully comments on the statements and conclusions set forth in the Examiner's Answer.

At page 6, the Examiner contends Raskin was not developing a new mechanism for plant tissues to produce protein, but merely in finding a cheaper way to isolate proteins by secretion (citing for example col. 2, ll. 29-50). Considering the Background of Raskin, proteins have been collected from media using cell cultures (col. 2, ll.1-19) and from wounds induced on transgenic rubber tree plants (col. 3, ll. 7-14 noting "tree must be damaged by wounding to recover heterologous polypeptide in the form of a latex mixture"). With respect to potato plants, recovery of heterologous HSA involved destruction of the plants (col. 3, ll. 1-6). Thus, Raskin concludes the need

existed to economically produce and recover heterologous polypeptides in quantity without destruction of the biological provider (col. 3, ll. 24-28). As such, Raskin developed a method to exploit the vascular system in higher order plants, which is absent in protonema, to retrieve heterologous polypeptides.

At pages 6 and 7, the Examiner argues Raskin is clear that plants can secrete heterologous proteins into media in which they are grown (citing col. 2, ll. 1-19) and discusses the use of hairy root cultures to produce antibodies secreted into and isolated from medium (citing col. 3, ll. 16-23). At page 9, the Examiner argues plant cell walls are permeable to proteins as large as 150 kDA, which includes antibodies (citing col. 2, ll. 1-19).

In response, col. 2, ll. 1-19 is clearly directed towards plant cell cultures and not intact plants. For instance, the passage begins at col. 1, l. 62,

> "Plant cells also reportedly produce chemicals such as native and heterologous polypeptides. Existing genetic transformation technologies allow the transfer of genes into a wide variety of plant cell lines... Further, plant

cell cultures have been shown to secrete low levels of heterologous proteins. Pen et al., Bio/Technology 10:292-296 (1992) reported that a bacterial signal sequence for alpha-amylase would direct secretion of this protein from tobacco cells. Thus, the plant cell wall has been shown to be permeable to polypeptides as large as 150 kDA. These tools have been used to reportedly engineer plant cells to produce and secrete a variety of heterologous polypeptides[.] Other polypeptides produced in plant cells include the Hepatitis B surface antigen (HBsAg), the enterotoxin B (LT-B) subunit from E. coli (a diarrhea inducer), a variety of antibodies, human growth factors, and hormones. Although these cells secrete the desired polypeptides, these systems also require energy and nutrient inputs that add to the costs of polypeptide recovery." (underlining added)

Appellants have addressed cell based technologies previously and rejections later withdrawn in regards to the comparison between intact organisms and cell cultures with respect to the cell wall. In review, cell cultures and whole

plant organisms differ due to different stages of differentiation and different cell wall structures. For example, the use of cell cultures as compared to protonema tissue was addressed in the Response to Office Action at pages 5-8, dated June 29, 2009, which incorporated the Neuhaus Declaration paras. 9 and 11-13 (also filed June 29, 2009), attached hereto in Appendix B. For instance, para. 9 provides,

> "Although cell suspensions such as NT-1 cells and BY2 cells are useful in studying biological processes such as those that affect cell division, the cytoskeleton and hormone signaling, experiments directed towards the cell wall itself including its function, its protective characteristics or its role as a barrier are not considered reflective of cells within the intact plant. This is due in part to the manipulation of the cell during the culture process to achieve its specialized characteristics."

Thus, experimental results obtained using cell cultures do not directly correspond to whole intact plants with regard to cell wall. With respect to the applicability of hairy root

cultures, Raskin provides at col. 3, ll. 20-23, "Further, some antibody activity was found in the medium of the hairy root cultures maintained under axenic conditions as heterotrophic biomasses require costly energy and nutrient inputs." Appellants first note hairy root cultures are not intact plants but are specialized cultures. Further, hairy root cultures are derived from roots, which include specialized cells used to overcome the barrier of the cell wall in higher order plants; however as emphasized throughout the Appeal Brief and Reski Declaration para. 47, protonema have no roots.

At page 7, the Examiner disagrees that Raskin is limited to root and leaf structures in higher plants as maintained by Appellant. Specifically, the Examiner argues plant portions, which may be parts of plants may be also used (citing col. 4, ll. 18-25) and argues at no point limits the material used to roots and leaves.

In response, while Raskin does state plant portions may be part or all of a plant organ or tissue, provided the material is intact and alive (col. 4, ll. 18-25), the plant portion would have to be consistent with collecting an exudate as set forth in the continuing paragraph (col.4, ll. 26-29), which summarizes

recovery of polypeptides,

> "In yet another aspect of the invention, the invention comprises recovering a polypeptide directly from a collected exudate, preferably guttation fluid, of a plant or a portion thereof. The recovery may be continuous."

The requirement that the plant exude produced polypeptides is also summarized in the abstract, "The methods of the invention use whole plants or portions thereof that are intact, living and capable of exuding the produced polypeptides." Thus, Raskin is limited to methods that utilize an exudate, which is characteristic of higher order plants and not protonema.

The plant exudate has been discussed previously as being oozed from roots or through the hydathodes of higher order plants (citing Reski Declaration para. 47). Collection of plant exudate for recovery of polypeptides is provided in Raskin's Examples 3-9.

Also at page 7, the Examiner argues the mechanism used in Raskin is endoplasimic reticulum-mediated secretion, using a signal peptide, and argues this secretion occurs in both moss

cells and in plants (offering Menon pg. 572, right col. para. 1). Indeed, in plants secretion does occur resulting in transfer between the cell membrane and the cell wall. For instance, referring to Menon at col. 2, ll. 1-4,

> "In this communication, we report on the relationship of the labeling kinetics of 14C-proline in the various cell wall fractions and the mechanism of secretion from the cytoplasm into the wall by the callus cells and gametophytes."

With respect to Menon generally, suspension cultures of callus cells or undifferentiated cells were studied and not intact mature protonema, which is identifiable in part by its filamentous morphology. For instance, referring to the abstract,

> "Electron microscopic evidence shows vesicular activity in the cytoplasm and secretion and incorporation into the wall layers (not shown). The protein probably functions in the maintenance of cell shape in the moss callus tissue, being

partly secreted out of the callus cells before the filamentous morphology appears." (underlining added)

Turning now to the passage on pg. 572 cited by the Examiner, experiments with callus cells and gametophytes showed three main results, namely a) callus cells secrete proteinaceous substances into the wall, b) under growth conditions some of the wall-protein diffuses out into the medium, and c) callus cells actively convert proline to hydroxyproline in comparison to gametophytes.

A first distinction is that again Menon relies on studies conducted with callus cells, which is a mass of undifferentiated cells and not protenema tissue as set forth in the claims. The use of cell cultures as compared to protonema tissue has been addressed previously as summarized above with respect to the Neuhaus Declaration.

With respect to diffusion of wall protein, Menon concluded that release of wall-bound protein was due to cellular differentiation. For example, referring to page 573, left col.,

"Since callus cells can differentiate both gametophytes and

apogamus sporophytes it can be concluded that the synthesis and release of the wall-bound protein is a consequence of the cellular differentiation."

For completeness, release of proteins in Menon occurred due to the loosening of the cell wall during development of the cell suspension. For instance, proceeding downward along page 573, left col.,

"Release of glycoproteins under high irradiance, from the suspension cultures of moss callus cells into the substrate was demonstrated in the present experiments. It is suggested that the shift in embryonal properties of the callus cells towards gametophytic development by cell wall loosening is brought about in high light intensities, and this changeover can be correlated with differential crosslinking of cell wall polysaccharides in the lateral wall and septum formation. *Physcomitrium* callus cells provide an opportunity to test the physiological regulation of this process."

Thus, this passage supports Appellants' continued position of why use of manipulated cell cultures or differentiating cultures would not be considered reliable for an intact plant with respect to the cell wall.

At page 9, the Examiner argues Raskin uses the term facilitated transport to mean secretion through the endoplasmic reticulum, a secretion system used in all eukaryotes and that ligand specificity and saturation kinetics are not involved in endoplasmic reticulum-mediated secretion.

For completeness, Appellants do not contend that protonema do not secrete protein through the plasma membrane to access the cell wall. However, the skilled artisan would not think the polypeptide will cross the cell wall to exit an intact organism.

The plant cell wall is a rigid, multi-layer structure consisting of diverse, highly organised polysaccharide fibrilles which are crosslinked and impregnated. (*see* Appendix B: FIGS. 1 and 2 with description, reproduced from Lodish et al, 2000). This gives plant cells a structural framework and to shield them from evaporation of their cellular fluids as well as from pathogen attacks. It is well known to one of ordinary skill in the art, that direct protein diffusion across the cell wall of

higher plants is unthinkable, due to the abovementioned rigidity and impregnation. In this context, it is not surprising that Raskin fails to give any evidence for such direct diffusion. In fact, all shown examples basically rely on specialised, locally restricted tissues or cells, whose task exactly is to overcome this barrier at a very restricted site. In accordance, Raskin explains several techniques to harvest the heterologous protein, which all aim to recover "exudate" or "guttation fluid" with the desired protein contained therein. As the tissues, namely hydathodes or the root epidermis, producing such "guttation fluids" or "exudates", do not exist in the protonema, Raskin does not apply to the claims.

Turning now to protein secretion, protein secretion refers to protein passage through the secretory pathway of, in this context, a eukaryotic cell, being initiated by the presence of a signal peptide at the N-terminus of the nascent protein. This process mechanistically relies in its last, the secretion-step, on a vesicular fusion of a secretory, protein loaded particle with the plasma membrane. (*see* Appendix B: FIG. 3 with description, reproduced from Lodish et al, 2000) Thus with respect to plants, secretion would presume to deliver the

protein to the cell membrane or plasma membrane but not across the cell wall. That is, vesicles do not fuse with the cell wall resulting in opening passages for release of its contents like that of the plasma membrane.

For completeness, neither diffusion nor facilitated transport is involved in secretion, nor is there any relevance in this argumentation to clarify the proteins passage across the plant cell wall of a developed plant.

2. Claim 22 remains rejected under 35 U.S.C. §103(a) as being unpatentable over Reutter et al. (1996, Plant Tiss. Cult. Biotechnol. 2:142-147) in view of Raskin (U.S. Patent No. 6,096,546) and further in view of Hein et al. (U.S. Patent No. 5,959,177). Appellants maintain that claim 22 is not obvious because Hein et al. do not correct the deficiencies of the rejection of Independent Claim 1 as previously argued and as shown above with respect to the use of cultured cells in col. 2, ll. 1-19 in the passage cited by the Examiner.

IV

Conclusion

It is respectfully submitted that all claims on appeal in this application are allowable. Accordingly, favorable consideration and reversal by the Honorable Board of Patent Appeals and Interferences of the Examiner's rejections under 35 U.S.C. §103(a) of claims 1-3, 17 and 22 are respectfully requested.

Respectfully submitted,

Feb 7, 2011

Date

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Attachments:
1) Appendices A-C
2) Declaration of Gunther Neuhaus filed June 29, 2009
3) Depictions of Excerpts from Lodish et al, Molecular Biology (Freeman Press 2000)

Appendix A

(Appendix of Claims Involved In the Appeal)

1. A method for production of heterologous proteinaceous substances in plant material, comprising the steps of:

culturing, in a culture medium, plant material transformed with a construct encoding a secretion signal peptide operably linked to a protein, that produces heterologous proteinaceous substances; and

obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells;

wherein the plant material is protonema tissue selected from the group consisting of *Physcomitrella patens, Marchantia polymorpha, Ceratodon purpureus,* and *Funaria hygrometica.*

2. The method according to claim 1, characterized in that proteinaceous substances released into the culture medium are biologically active.

3. The method according to claim 1, characterized in that the culture medium is free from sugars, vitamins and phytohormones.

17. A method for the production of heterologous proteinaceous substances in plant material, comprising the steps of:

culturing, in a culture medium, photosynthetically-active plant material transformed with a construct encoding a secretion signal peptide operably linked to a protein, that produces heterologous proteinaceous substances; and

obtaining secreted heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells,

wherein the photosynthetically-active plant material is protonema tissue selected from the group consisting of *Physcomitrella patens, Marchantia polymorpha, Ceratodon purpureus,* and *Funaria hygrometica.*

22. The method according to claim 1, characterized in that the proteinaceous substances are antibodies capable of specific binding with antigen.

24. The method according to claim 1, characterized in that the proteinaceous substances are enzymes capable of converting a target substrate to product.

Appendix B

(Evidence Appendix)

A copy of evidence pursuant to 37 CFR § 1.132 (a Declaration by Gunther Neuhaus) that is relevant to this appeal is attached hereto. The executed Rule 132 Declaration was entered on June 29, 2009.

Depictions of secretion pathway of protein, the plant cell wall and the structure of the secondary plant cell wall are provided for pictorial reference of secretion and cell wall components. Reproduced from Lodish et al., Molecular Cell Biology. 4 Ed. New York: W.H. Freeman, 2000 (ISBN 0-7167-3136-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)
	)
Gilbert GORR et al.	)
	)
Serial No. 10/089,450	) Group Art Unit: 1638
	)
Filed: March 29, 2002	) Examiner: KUBELIK, Anne R.
	)
For: METHOD FOR THE PRODUCTION OF PROTEINACEOUS SUBSTANCES	)

DECLARATION UNDER 37 C.F.R. § 1.132 BY GUNTHER NEUHAUS

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

1. I, Gunther Neuhaus, state that I am an expert in the field of plant cell cultivation research and development. I hold a PhD in Biology (Botany, Zoology) and am a professor of Cell Biology at the University of Freiburg. A copy of my Curriculum Vitae is attached herewith as evidence of my relevant expertise.

2. I am familiar with the above-captioned patent application and claims. In order to appreciate biological differences and predicted activities between cells isolated from vascular plants, such as tobacco cells and whole protonema tissue, in this declaration, I review relevant literature.

3. A bryophyte is a non-vascular plant, which generally means it does not have a vascular system (xylem and phloem). Bryophytes do not have flowers and do not

produce seeds. They have enclosed reproductive systems and reproduce via spores. The bryophyte life cycle progresses from spore to protonema to gametophore.

4. *Nicotania tobacum* is commonly referred to as the tobacco plant. Tobacco plants are vascular in nature. Vascular plants are distinguished in part by the presence of vascular tissues (xylem and phloem), which circulate resources throughout the plant. The tobacco plant produces seeds and flowers.

5. In intact plants, plant cells are surrounded by a cell wall. The cell wall is a tough rigid layer that protects plant cells from environmental conditions, which can be highly varied. Among these include light conditions such as content of UV or the broad variability in air humidity.

6. Although the cell wall provides a rigid outer barrier, plant cells are interconnected by plasmodesmata. Plasmodesmata are pores through the cell wall, by which individual cells may be interconnected by membranes, cytoplasm and by the endoplasmatic reticulum. This is essential for a vascular plant as there are cells without nuclei existing, which receive needed nutrients and expression products from neighboring cells (accompanying cells support the sieve elements by this method in the phloem).

7. There are different approaches to studying biological processes in plants. Among these include studying the intact plant itself and studying plant cells such as cell

suspensions derived from intact plants. Whether to perform experiments on intact plants or cell suspensions may depend on the process studied.

8. Many plant experiments are performed using cell suspensions. Among these including *Nicotania tobacum* clone-1 cells ("NT-1 cells") and Bright Yellow 2 cultivar of the tobacco plant ("BY2 cells"). In suspension NT-1 and BY2 cells float independently or in groups but the suspension itself is not interconnected by plasmodesmata as found in intact plants. While cell suspensions do not retain many of the characteristics of cells of an intact plant, many biological processes such as cell division, cytoskeleton and hormone signaling may be studied using cell suspensions.

9. Although cell suspensions such as NT-1 cells and BY2 cells are useful in studying biological processes such as those that affect cell division, the cytoskeleton and hormone signaling, experiments directed towards the cell wall itself including its function, its protective characteristics or its role as a barrier are not considered reflective of cells within the intact plant. This is due in part to the manipulation of the cell during the culturing process to achieve its specialized characteristics.

10. To better understand the manipulation of the plant cell during its transformation from intact plant to a cell suspension, I provide the following overview of the processes.

11. In vitro cell cultures from vascular plants are initiated from sterilized organ explants. These initial explants are induced to form an undifferentiated callus (cell mass). The cells in this callus lose typical plant and organ specific expression (such as expression of photosynthetic genes and several metabolic genes) and only express so called housekeeping genes. As such these callus cells also lose over time the potential for regenerating a complete plant. These cells are considered specialized cells and are so called "habituated plant cells."

12. To establish a suspension culture from the specialized callus cells, several steps have to be followed. First, the callus has to be broken down in small mostly single cell aggregates which have to be cultured in a highly complex liquid culture medium including vitamins, sugars as well as plant hormones. Afterwards the cell suspension has to be subcultured every 8 to 10 days to ensure continuous cell division. Upon this subculturing procedure the cells have to be sieved, so that mostly only single cells or at least small cells serve as starting culture for the next growing cycle. If this is not done in the appropriate way the cells will die in the old suspension culture upon nutrient deficiency. In addition if the cell aggregates are grown too big they also will die as the inner cell mass will not get the required nutrients.

13. There is a high heterogeneity in the starting plant cell suspensions. Further culturing is performed to eliminate this heterogeneity. Thus, the specialized cell suspension is adapted for liquid culture conditions. Among the adaptations, especially with respect to homogeneity in "humidity" there is no need for a strong barrier i.e. rigid

cell wall against the environment surrounding medium. In addition, cell suspensions such as NT-1 and BY2 cells have also been cultured over years and selected for additional special selected features (e.g. synchronized cell cycle in tobacco BY2 cell cultures).

14. Once specialized into NT-1 or By2 cell suspensions, structural changes in their cell biology appearance and in particular the cell wall is evident. For instance, whereas cells in whole intact vascular plants are interconnected by plasmodesmata, the suspension of NT-1 and BY2 cells are not interconnected. Instead, NT-1 and BY2 cells are typically found floating independently or in small groups.

15. Functionally, NT-1 and BY2 cells obtained from suspension compared to cells provided within the native intact vascular plant behave differently. This can be evidenced in part by taking plant cells from a cell suspension and culturing them on an agar medium under same conditions in which sterile in vitro plants or plant cuttings can be grown easily. Within one week all cells from the suspension culture stop their division capacity and due to their artificial nature caused by their culturing technique (in suspension) they will die after one week.

16. Since the barriers of NT-1 cells and BY2 cells are known to be manipulated to facilitate culturing in suspension, it would not be logical to study the role or characteristics of a plant cell wall using NT-1 cells or BY2 cells. Instead, one would logically study whole intact tissue or a whole intact plant. Thus, a comparison between

effects observed in a culture of suspended specialized cells would be difficult to transfer to an intact plant.

17. A comparison between the cultivation of differentiated bryophytes or differentiated tissue thereof in liquid culture, in which the differentiated non-vascular plant gametophyte or protonema is cultivated, and a suspension culture of specialized plant cells derived from vascular plants is scientifically very difficult. In one case the cultured material is the whole differentiated organism (bryophyte) or differentiated tissue thereof like protonema whereas in the other case a selected artificial and undifferentiated sporophytic cell suspension is used. The difference is mostly obvious when explants will be taken from both suspension cultures and plated on agar. As bryophyte material from suspension culture represents a whole organism or tissue thereof - in both cases the differentiated cells are highly regenerative - it will grow and develop gametophytic fully developed organisms, whereas a cell from the sporophytic cell suspension like BY2 or NT-1 cell cultures will perform in the ideal case one or two cell divisions and then slowly will die under these conditions, but never develop - even under the best nutrients - to a whole sporophytic organism.

18. There exists also in higher plants gametophytic cell suspension cultures which are able to regenerate to non fertile plants, but the culture conditions are very limited. First the explant material is immature gametophyte (microspores) just after meiosis, secondly these cultures are genetically very limited, as they have very low expression activity and thirdly the cultures have to be initiated for regeneration within

short time (1 – 2 month) as they lose their regeneration capacity very fast and develop into slow dividing and finally dying cells. Moreover the cell wall of this gametophytic plant material is different from that of all other vascular plant cells, as their normal development is primed to be a mature gametophyte, which is a pollen, that has to survive rough environmental conditions such as drought and high/low temperatures (this is made by deposition of lipophil material within the cell wall to avoid any loss of water and thereby also any secretion of water soluble compounds like salts into the surrounding media). By this the pollen (the gametophyte of vascular plants) contains the most thick and hardest cell wall that can be found in the plant kingdom.

19. In conclusion from a point of a cell biologist, a direct comparison between a moss suspension culture containing whole gametophyte or tissue thereof (like protonema) and a suspension of specialized cells from vascular plants like NT-1 and BY2 is therefore inadequate and biological conclusions -especially regarding the barrier e.g. cell wall system against the outer environment- are as useless for transfer to the whole gametophyte or tissue thereof (like protonema) of liquid bryophyte cultures and vice versa.

20. I declare under penalty of perjury that the foregoing is true and correct, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code,

and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed by,

Date: 26.06.09

Name: Gunther Neuhaus

CV: Prof. Dr. Gunther Neuhaus

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Grades and Post Graduate Studies

1974	Gymnasium Linz / Austria

University

1974-1978	Study (Biology) at the University Salzburg (Austria) (Mag. rer. nat.)
1980	Graduation for Dr. phil. in Cell Biology and Botany (University Salzburg, Austria)
1993	Habilitation at the ETH-Zürich ("New approaches in plant development"), Venia legendi „Plant Developemnt"

PROFESSIONAL EXPERIENCE

1980	Research Assistant at the University Salzburg (Austria), Institute for Plant Sciences
1980-1982	Postdoc at the Max-Planck-Institute für Cell Biology, Ladenburg/Heidelberg, Germany
1982	6 month stay as Visiting Scientist at the Rockefeller University, New York with Prof. Dr. N.-H. Chua, USA
1982-1987	Research Assistant at the Max-Planck-Institute for Cell Biology, Ladenburg/Heidelberg, Germany
1987-1993	Assistant Professor at the Institute for Plant Sciences at

	the ETH-Zürich, Switzerland (Swiss Federal Institute of Technology)
1993-1996	Visiting Associate Professorship at the Rockefeller University, Department of Plant Molecular Biology, Prof. N.-H. Chua (New York, USA)
since 1995	Head of Department, Institute of Cell Biology, University Freiburg, Germany
since 1998	Managing Director oft he Center of Applied Sciences University Freiburg, Germany
2002	Founder of the Biotech Company "greenovation", Freiburg, Germany (together with Prof. Reski)
Since 2002	Member of the "BioValley Expert" Teams
2002	Member of the "Task force group in Life Science" of the University Freiburg
since 2003	Advisor "Biotechnology-Team Baden-Württemberg"
since 2003	Elected Member of the "Strasburg-Author team"

Awards

1980	1. Preis der Stadt Salzburg für die beste Doktorarbeit.

Elected Memberships

2000	Deutsche Akademie der Naturforscher „Leopoldina" (German Academy of Science)

Referee

For international Organizations DFG, NSF, USDA, Japanese Frontier Science Program, Swiss Nationalfonds, Human Science Frontier Program Organization, und other Organizations.

Journals - Plant Physiology, Plant Cell, Plant Cell Physiology, Cell, Nature, EMBO J., Science, MGG, Plant Cell & Environment, Plant Mol. Biology, Planta, etc.

Book Authorship:

Strasburger - Lehrbuch der Botanik: Bresinsky, A., **Körner**, C., **Kadereit**, J.W., **Neuhaus**, G., **Sonnewald**, U. 36. Aufl., 2008, XVI, 1176 S. 957 Abb., 465 in Farbe., Geb. ISBN: 978-3-8274-1455-7

Freiburg, 2.2.2009 ***Gunther Neuhaus***

Prof. Dr. Gunther Neuhaus

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The Plant Cell Wall

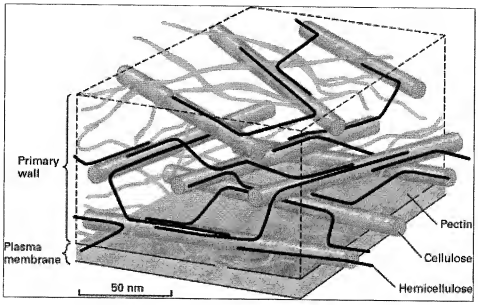

FIG. 1: Schematic representation of the pimary cell wall of an onion. Cellulose and hemicellulose are arranged into at least three layers in a matrix of pectin polymers. The size of the polymers and their separations are drawn to scale. To simplify the diagram, most of the hemicellulose cross-links are not shown. [Adapted from M. McCann and K. R. Roberts, 1991, in C. Lloyd, ed., The Cytoskeletal Basis of Plant Growth and Form, p. 126.] From *Lodish et al* 2000

Structure of the Secondary Cell Wall

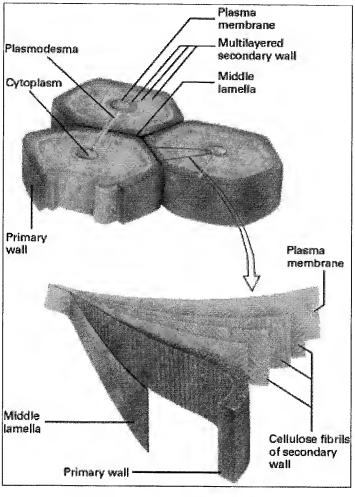

FIG. 2: The structure of the secondary cell wall, built up of a series of layers of cellulose. In each layer, the cellulose fibrils run more or less in the same direction, but the direction varies in different layers. As plant cells grow, they deposit new layers of cellulose adjacent to the plasma membrane. Thus the oldest layers are in the primary wall (the outer wall) and in the middle lamella (the pectin-rich part of the cell wall laid down between two daughter cells as they cleave during cell division). Younger regions of the wall—collectively the secondary cell wall —are laid down as successive layers, adjacent to the plasma membrane. The cytoplasms of adjacent cells are usually connected by plasmodesmata that run through the layers of the cell walls. From *Lodish et al, 2000*

Secretory Pathway to the Plasma Membrane

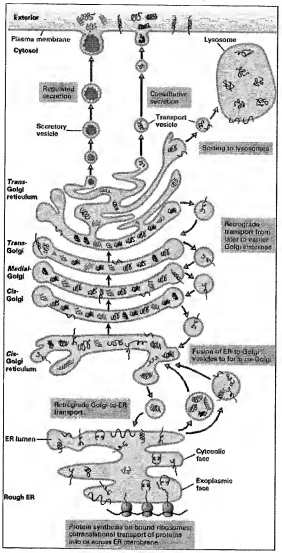

FIG. 3: The secretory pathway of protein synthesis and sorting. Ribosomes synthesizing proteins bearing an ER signal sequence become bound to the rough ER. As translation is completed on the ER, the polypeptide chains are inserted into the ER membrane or cross it into the lumen. Some proteins (e.g., rough ER enzymes or structural proteins) remain resident in the ER. The remainder move into transport vesicles that fuse together to form new cis-Golgi vesicles. Each cis-Golgi cisterna, with its protein content, physically moves from the cis to the trans face of the Golgi stack (red arrows). As this cisternal progression occurs, many luminal and membrane proteins undergo modifications, primarily to attached oligosaccharide chains. Some proteins remain in the trans-Golgi cisternae, while others move via small vesicles to the cell surface or to lysosomes. In certain cell types (e.g., nerve cells and pancreatic acinar cells), some soluble proteins are stored in secretory vesicles and are released only after the cell receives an appropriate neural or hormonal signal (regulated secretion). In all cells, certain proteins move to the cell surface in transport vesicles and are secreted continuously (constitutive secretion). Like soluble proteins, integral membrane proteins move via transport vesicles from the rough ER to the cis-Golgi and then on to their final destinations. The orientation of a membrane protein, established when it is inserted into the ER membrane, is retained during all the sorting steps: Some segments always face the cytosol; others always face the exoplasmic space (i.e., the lumen of the ER, Golgi cisternae, and vesicles or the cell exterior). Retrograde movement via small transport vesicles retrieves ER proteins that migrate to the cis-Golgi and returns them to the ER. Similarly, cis- or medial-Golgi proteins that migrate to a later compartment are retrieved by small retrograde transport vesicles. [See B. Glick and V. Malhotra, 1988, Cell 95:883.] From *Lodish et al, 2000*

Appendix C

(Related Proceedings Appendix)

None